Discovering Ionic Liquid Resistant Genes

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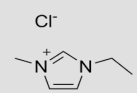
1

Abstract: Plant biomass is a rich source of sugars that can be converted to biofuels by engineered microbes. However, because the lignocellulose in biomass is insoluble in aqueous conditions and recalcitrant to enzymatic degradation, thermochemical treatment is required to break apart the lignin and cellulose polymers before sugars can be released. The most effective chemicals for doing this are known as ionic liquids, which are salts that are molten at temperatures below 100° C. Although these solvents have many unique properties that are ideal for solubilizing lignocellulose, they have been found to inhibit the growth of bacterial strains used to produce biofuels. We therefore searched for molecular mechanisms in bacteria that enable normal growth in the presence of ionic liquids and that can be engineered into our laboratory strains. To approach this, we are screening many environmental isolates as well as complex metagenomic DNA samples for ionic liquid resistance genes. Our initial studies have resulted in several genes that hold great promise for increasing the efficiency of microbial biofuel production by constructing ionic liquid tolerant strains of *E. coli*.

2

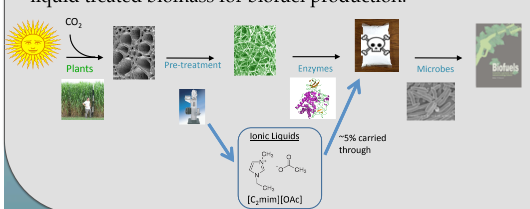
Introduction:

Problem: Chemical Toxicity: Ionic liquids are molten salts used to solubilize and separate the cellulose and lignin components of biomass. The ionic liquid salts have proven to be among the most effective treatment processes to achieve this goal. However, their toxicity to biofuel producing microbes has become a critical barrier in this process. This toxicity is a critical barrier to the use of ionic liquid treated biomass for biofuel production.



1-ethyl- 3-methyl imidazolium chloride (ionic liquid)

Screening an Environmental Metagenome: To overcome the problem of ionic liquid toxicity, we wanted to engineer tolerance into our biofuel microbes. By screening environmental DNA collected from soils in the Amazon Rainforest, we hoped to identify ionic liquid resistance genes.



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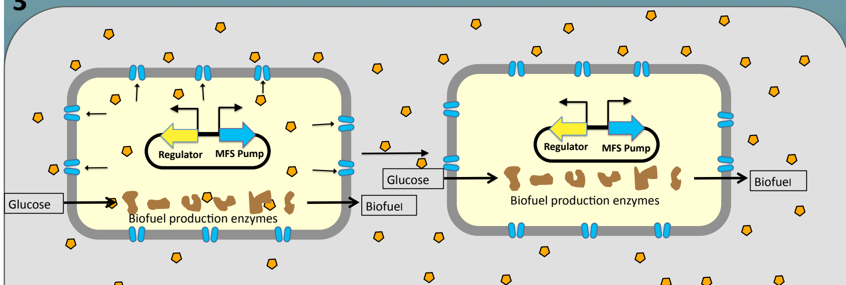
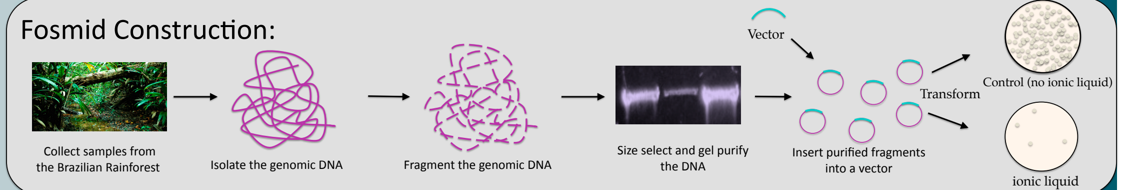


Previous research at JBEI has identified membrane transporters known as efflux pumps that relieve the bacterial strains from IL-mediated growth inhibition. This particular pump is a member of the Multifacilitator Superfamily (MFS) of proteins and its expression is regulated by an adjacent transcription factor. When exposed to ionic liquids, the pump exports them from the cell, promoting normal growth.

4

Fosmid Construction:



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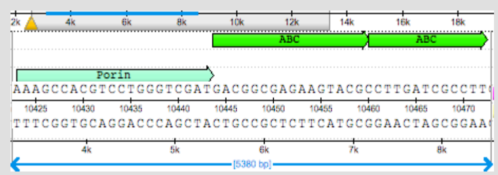
Targeted Approach

Fosmid	Possible Gene
1	MFS pump
2	Drug transporter
3	ABC pump
4	Pump transcriptional regulator & ABC Pump

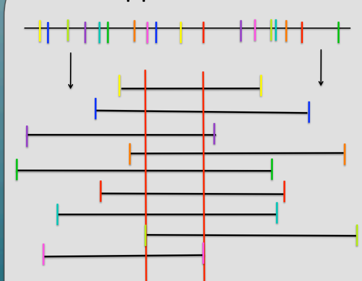
Fosmid 3 Candidate Gene

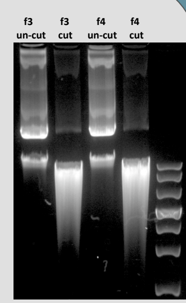


After the screening the successful fosmids were sequenced. The fosmid sequences were examined for possible resistance genes. Candidate genes were cloned and further analyzed.

6

Broad Approach

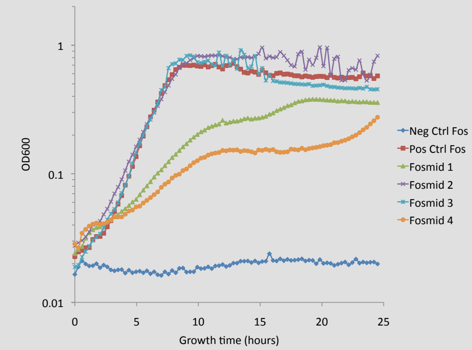




DNA from fosmids 3 and 4 was sheared into 6-8 kb fragments, (See gel image). We are now screening these fragments to determine which regions of the fosmid (i.e., between the vertical red lines) are responsible for its resistance phenotype.

7

Fosmid Growth Curve in Ionic Liquid



Growth of E. coli containing our newly identified fosmids (1-4) in 250 mM ionic liquid medium. Fosmids shown in comparison with control cells (blue) lacking a fosmid and a positive control containing a tolerant pump (red).

8

Conclusion

- Fosmids 2 and 3 are as tolerant as the positive control, while 1 and 4 protect more modestly.
- Fosmid sequencing analysis shows that a variety of transporter genes and transporter regulators are contained in our fosmids.
- There is strong reason to believe that an MFS pump, responsible for tolerance in previous research findings, is responsible for the tolerance phenotype of fosmid 1.
- Fosmids 2, 3, and 4 are all novel as they do not contain any transporters currently known to transport ILs. Their protective effects are likely due to the presence of either other classes of transporters or of genetic regulators that activate existing *E. coli* genes.

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